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PHENOLIC COMPOUND AND ANTIOXIDANT POTENTIAL OF *Hebeloma sinapizans* MUSHROOM

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Abstract

Mushrooms have been used for both culinary and medicinal reasons for a very long time. Mushrooms are useful as both a food and a medication due to their high nutritional, protein, vitamin, and phenolic content. The current research aims to determine the antioxidant activity and phenolic content of Hebeloma sinapizans. Multiple epidemiological research looked at the possible link between polyphenol consumption and cardiovascular illness. Antioxidant, antiplatelet, antiinflammatory, antilipidemic, and antihypertensive are the categories used to classify the actions of phenolic substances. HPLC was used to isolate the phenolic compounds (HPLC). Measurements of oxidative stress, total oxidant levels, and total antioxidant levels were all calculated with the use of Rel Assay kits. The mushroom's TAS, TOS, and OSI values were measured, and its gallic acid, chlorogenic acid, and cinnamic acid concentrations were identified. Due to its high levels of phenolic compounds, H. synapizans is thought to be a source of phenolic compounds. High TAS and TOS values and low OSI values indicate that the mushroom has antioxidant potential. As a result, it has been seen that H. synapizans can be a good source of antioxidants.

Key words: *Antioxidant, bitter bolet, medicinal mushroom, oxidant, wild mushroom.*

INTRODUCTION

Humans have harvested mushrooms from the wild for a variety of reasons and used them for hundreds of years. Mushrooms are widely used all over the globe as a source of food due to its high nutritional, protein, and vitamin content. (Vidović et al., 2010; Sevindik et al., 2019). Mushrooms have been studied for their medicinal properties, which include antibacterial, antiviral, antitumor, antioxidant, anti-inflammatory, and immune-boosting properties in addition to their dietary benefits. (Mau et al., 2002; Tian et al., 2012; Jo et al., 2013; Sevindik et al., 2018; Bal et al., 2019; Islam et al., 2019). Mushrooms have an important function in the breakdown of organic materials, in addition to its dietary and therapeutic benefits. Mushrooms are known to possess antioxidant capabilities, which play a significant role in reducing the impact of oxidative stress on living organisms (Klaus et al., 2019). Diseases of the nervous system, the heart, the liver, diabetes, leukemia, senescence, and cell death are all linked to free radicals and the oxidative stress they create (Choi et al., 2006; Babu et al., 2013; Korkmaz et al., 2018; Mushtaq et al., 2020).

Numerous variables contribute to cardio-vascular disease's complexity and multifaceted nature. Heart disease risk factors (hypertension, diabetes mellitus, high cholesterol, smoking, race/ethnicity/gender/age) are listed in (Rahman and Lowe, 2006). Worldwide, many people die each year due to cardiovascular diseases (Reddy & Yusuf, 1998; Gersh et al., 2010). Depending on the diet content of people, the rate of catching diseases varies. Consumption of foods high in phenolic content is important in combating diseases (Wang et al., 2012). It has been reported that there is a link between the consumption of foods high in phenolic compounds and cardiovascular diseases. In this context, it has been observed that there is a decrease in the incidence of myocardial infarction, especially with the consumption of diets high in polyphenols. It has also been reported that phenolic compounds improve cardiovascular risk factors such as hypertension, endothelial dysfunction, lipid metabolism and platelet activation (Peters et al., 2002). It is also known that phenolic compounds have antioxidant, antiplatelet, anti-inflammatory, antilipidemic and antihypertensive effects (Aviram et al., 2000; Garcia-Lafuente et al., 2009).

H. sinapizans is a mushroom that forms mycorrhiza on hardwood and conifers in late summer and autumn. It spreads cosmopolitanly. As a result of the consumption of the mushroom, gastrointestinal syndrome is observed. Excessive consumption can cause serious damage to the kidneys (O'Reilly, 2011). The aim of this study is to determine the potential for use of *H. synapizans* mushroom in pharmacological studies. In this context, antioxidant and oxidant levels of the mushroom were determined. In addition, phenolic compounds in its composition were screened. Depending on the phenolic compounds detected in its content, its potential in preventing cardiovascular disorders was evaluated.

MATERIALS AND METHODS

H. synapizans samples used in this study were collected from Antalya, Turkey. Fungarium samples of mushroom are kept in the fungarium of Akdeniz University, Department of Biology. First, the mushroom was dried in an incubator at 40°C. After the sample was dried, it was pulverized in a machine. After grinding, 30 g of sample was extracted with ethanol (Sigma-Aldrich) at 50°C for approximately 6 hours in the extractor. The solvents of the extracts (BUCHI Rotavapor Model R-144) were removed using a rotary evaporator.

Determination of phenolic content

The extracted mushroom samples were processed using the technique described by Caponio et al. (1999), with some minor modifications, employing SHIMADZU system HPLC and DAD detector, to determine the phenolic contents of the supplied mushrooms. The volume of the injection was changed to 20 L. The flow rate was set at 0.8 mL/min, with mobile phase A consisting of 3% acetic acid and mobile phase B consisting of methanol. At 30°C, we used a 250 x 4.6 mm internal diameter (5 M) Agilent Eclipse XDB-C18 column for chromatographic separation.

Antioxidant and oxidant tests

Rel Assay brand kits were used to measure the TAS and TOS levels in the mushroom extracts (Rel Assay Kit Diagnostics, Turkey). The TAS values were determined by using Trolox as a calibrator, and the findings were expressed as mmol Trolox equiv./L. The TOS values were calculated by using hydrogen peroxide as a calibrator, with the final findings expressed as micromoles of hydrogen peroxide equivalent per liter (mol H_2O_2 equiv./L) (Erel, 2004; 2005). The OSI value was determined by dividing the TOS value of the mushroom with the TAS value and taking the percentage. (Sevindik, 2018).

$$\text{OSI (AU)} = \frac{\text{TOS, } \mu\text{mol } H_2O_2 \text{ equiv./L}}{\text{TAS, mmol Trolox equiv./L X 10}}$$

Statistical analysis

Means StD (standard error of the mean) of triplicates were reported for the experimental findings. Microsoft Office 2016's Excel statistical capabilities were used to examine the data. A level of significance of $p<0.05$ was used to identify differences.

RESULTS AND DISCUSSIONS

Phenolic content

The phenolic compounds of *H. sinapizans* were determined using HPLC and the findings are shown in Table 1.

Table 1. Phenolic compounds in *H. sinapizans*

	Gallic acid	Chlorogenic acid	Cinnamic acid
H. sinapizans	1.71 ppm	40.01 ppm	1.32 ppm

In our study, gallic acid, chlorogenic acid and cinnamic acid were detected in the mushroom as a result of the analyses. The phenolic chemicals have numerous biological functions, one of which is antioxidant action (Saxena et al., 2012). Gallic acid, also known as 3,4,5-trihydroxybenzoic acid, is a very powerful polyphenol with notable antioxidant properties. Gallic acid has antioxidative capabilities that prevent lipid peroxidation in erythrocyte membranes and microcomes and reduce the carcinogenic effects of hydrogen peroxide, according to a recent study (Shiraki et al., 1994; Badhani et al., 2015). Due to its strong antioxidant properties, gallic acid prevents some of the damage caused by myocardial infarction (Priscilla & Prince, 2009). Myocytes may be protected from free radical damage by antioxidant activity (Priscilla & Prince, 2009; Kasperet al., 2015). Gallic acid's effects on vascular calcification, cardiac hypertrophy and fibrosis, hypertension, and oxidative stress have been seen in a few research investigations (Kee et al., 2014; Ryu et al., 2016; Jin et al., 2017). Unfortunately, heart failure is one of the most frequent cardiovascular disorders. Reduced ventricular compliance, remodeling of the chambers, and interstitial fibrosis are hallmarks of heart failure. Gallic acid is effective in treating heart failure, which is linked to cardiac hypertrophy and hypertension. Chlorogenic acid is a dietary supplement used for its blood-pressure-lowering, anti-inflammatory, chemical-sensitizing, and properties (Kwon et al., 2010; Zhao et al., 2012; Onakpoya et al., 2012; Tajik et al., 2017). Chlorogenic acid may help with diabetes, hypertension, tumors, cholesterol, inflammation, and oxidative stress (Suzuki et al., 2010; Ong et al., 2012). All of these things are well-known to reduce the risk of cardiovascular disease. In addition, chlorogenic acid has been linked to a reduction in the risk of cardiovascular disease due to its antiplatelet action (Amin et al., 2013; Bijak et al., 2013). The precise processes by which this protection is achieved are still being investigated. It has been shown that chlorogenic acid prevents thrombus formation *in vivo*, decreases platelet production of inflammatory mediators linked to atherosclerosis, and contains antiplatelet action (Ji et al., 2011).

Natural low molecular weight phenolic compounds like cinnamic acid have anti-microbial and anti-oxidant properties (Cruz et al., 2005). Numerous studies have found that cinnamic acid has beneficial effects, including those on inflammation, oxidation, tumors, bacteria, and multiple cytoprotective actions that reduce neuroinflammatory symptoms in degenerative diseases. It also lowers blood pressure and cholesterol levels by inhibiting the oxidation of low-density lipoprotein (LDL) (Nardini et al., 1999; Zang et al., 2000; Pontiki et al., 2006; Naz et al., 2006; Szwajgier et al., 2017). Obese rats treated with cinnamic acid had considerable weight loss and ACE activity in their blood was dramatically reduced (Mnafgui et al., 2015). It also has a cardioprotective profile due to its beneficial effects on vasoconstriction and hypertension problems (Mnafgui et al., 2015).

Based on the evidence presented, it is possible to conclude that the phenolic compounds extracted from the *H. sinapizans* mushroom can make a significant contribution as anti-oxidants, and that further research into the extraction of other important phenolic compounds and their biological effects is warranted.

TAS, TOS and OSI values

The free radicals produced by living organisms, as well as the environmental and intrinsic influences on them, need the employment of antioxidant chemicals to mitigate their destructive potential (Mohammed et al., 2020; Bal et al., 2023). The body naturally produces these chemicals, which act to neutralize free radicals. In cases when naturally occurring antioxidant chemicals are insufficient, it is crucial to supplement the diet with exogenous antioxidants in order to reduce and prevent oxidative damage (Selamoglu et al., 2020; Uysal et al., 2023). In order to discover novel sources of antioxidants, it is crucial to analyze the mushroom's antioxidant activity and oxidative stress index (Bal et al., 2021). The TAS (mmol/L), TOS (mol/L), and OSI values of *H. sinapizans* were measured using the prescribed procedure and are shown in Table 2.

Table 2. TAS, TOS and OSI values of *H. sinapizans*

	TAS	TOS	OSI
H. sinapizans	4.540 ± 0.113	10.303 ± 0.050	0.227 ± 0.006

In previous studies on wild mushrooms, the TAS value of *Ramaria stricta* was reported as 4.223 mmol/L, the TOS value as 8.201 µmol/L and the OSI value as 0.194 (Krupodorova & Sevindik, 2020). The TAS value of *Lactifluus rugatus* was reported as 3.237 mmol/L, the TOS value as 8.178 µmol/L and the OSI value as 0.254 (Sevindik, 2020). The TAS value of *Tricholoma imbricatum* was reported as 3.474 mmol/L, the TOS value as 15.257 µmol/L and the OSI value as 0.439 (Bal, 2021). The TAS value of *Cyclocybe cylindracea* was reported as 4.325 mmol/L, the TOS value as 21.109 µmol/L and the OSI value as 0.488 (Sevindik et al., 2018). The TAS value of *Fomitopsis pinicola* was reported as 1.44 mmol/L, the TOS value as 14.21 µmol/L and the OSI value as 0.99 (Sevindik et al., 2018). Compared to these studies, the TAS value of *H. sinapizans* was found to be higher than *R. stricta, L. rugatus, T. imbricatum, C. cylindracea* and *F. pinicola*. The TAS value shows the whole of the antioxidant compounds produced in living organisms (Sevindik et al., 2018). These findings demonstrated that *H. sinapizans* is rich in antioxidant compounds that could neutralize oxidant molecules and could produce more antioxidant molecules compared to others. High TAS value of *H. sinapizans* obtained in the present study indicated that this mushroom could be used as a rich natural antioxidant source.

TOS value of *H. sinapizans* was lower than *R. stricta* and *L. rugatus* and higher than *T. imbricatum, C. cylindracea* and *F. pinicola*. The TOS value shows the whole of oxidant compounds produced by environmental effects in living organisms (Sevindik et al., 2018). According to these results, it was determined that the TOS value of *H. synapizans* was at normal levels compared to the mushroom reported in the literature. The OSI value of *H. sinapizans* was higher than *R. stricta*, lower than *L. rugatus, T. imbricatum, C. cylindracea* and *F. pinicola*. The OSI value indicates how much endogenous antioxidants suppress endogenous oxidants produced in living organisms. The lower the OSI value, the better the antioxidant defense system (Sevindik et al., 2018). In this context, it is seen that *H. synapizans* suppresses endogenous oxidant compounds well with antioxidant compounds produced in its body.

CONCLUSIONS

In this study, antioxidant activity and phenolic content of *H. sinapizans* were determined. These data suggest that the mushroom *H. synapizans* is toxic to humans but could be used as a potential antioxidant for future pharmacy. These studies suggest that *H. sinapizans* may exhibit potential antioxidant activity if extended to identify other potent phenolic compounds and use them for pharmacological effects. Its cardioprotective effect with the phenolic compounds it contains can be used in the treatment of cardiovascular diseases in the future. In addition to all these, since the mushroom is poisonous, its consumption should not be made directly. However, the compounds identified in its structure may be isolated in future studies and their pharmacological use may be valuable.

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